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Fumigant toxicity of callistemon viminalis essential leaves oils against vinegar fly, Drosophila melanogaster

Hind Suhail Abdulhay

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

E-mail : hind442@yahoo.com Contact No. : 009647716687403

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Abstract

The essential oils of *Callistemon viminalis* (Gaertn) were obtained using a Clevenger type apparatus through hydrodistillation. The essential oils were tested for fumigant toxicity against adults (1-3 day old) of *Drosophila melanogaster*. All toxicity tests were carried out under laboratory conditions set at 23 ±2 ºC and 60% relative humidity (RH). Results showed that tested concentrations exhibited high toxicity effects on *D. melanogaster* adults and the high toxicity rate was concentration and time dependent. Lethal concentration values for 50% of *D. melanogaster* adults (LC_{50}) were 56.57, 37.22 and 20.58 µl/L air, after 5, 10 and 24 h. respectively. The results of gas chromatography (GC) analysis indicated that the essential oil extracted from the leaves of *C. viminalis,* contain 1,8-cineole (57.35%), *α*-pinene (10.20%), aspartic acid (12.30%) and palmitic acid (15.65%). These results suggest that *C. viminalis,* oil could be a potential candidate as a fumigant for controlling vinegar fly *D. melanogaster.*

Key words : *Callistemon viminalis, Drosophila melanogaster,* Essential oils, Insecticidal activity.

INTRODUCTION

Drosophila melanogaster, commonly known as vinegar or fruit fly, is a species of fly belongs to the order Diptera in family Drosophilidae. The species are small, slow-flying insects usually found in association with over-ripened fruit and vegetables. Vinegar flies are most abundant as common nuisance pests in grocery stores, fruit markets, restaurants, canneries, homes, and other occupied places where tomatoes, apples, and fruit may attract these insects with fermenting or rotting vegetative matter. Worldwide, the family Drosophilidae has over 3,000 described species in about 60 genera. The genus *Drosophila* contains more than half of the known species-most of these are found in the tropics. The wide range of *D. melanogaster* habitats and survival are mainly limited by low temperature and the lack of water. Therefore, *D. melanogaster* is found in almost every continent of the world except Antarctica but the distribution of *D. melanogaster* is changing with the worldwide climate changes [1].

Recently, an interest in natural products from plants has been increased due to the disruption of natural biological control systems, undesirable effects on non-target organisms, environmental hazards, and the development of resistance to synthetic insecticides, which are applied in order to reduce the populations of insects [2,3].

Fumigation plays a very important role in insect pest elimination in products [4]. Since botanical insecticides have low toxicity, naturally available plant materials, biodegradable and safe for managing and control insects. Many studies isolated and identified several chemical compounds from different parts of plants and screened out for growth inhibitors and insect deterrents [5].

Callistemon viminalis (commonly known as weeping Bottlebrush) is a small tree or shrub with pendulous foliage; also, some forms are more pendulous than others. It reaches a height of about 4 m in its natural habitat, but is usually smaller in cultivation, particularly in temperate areas [6]**.** Previous studies have investigated the potential effect of *Callistemon* specie such as [7] which used *Callistemon* as weed control. And [8] used *Callistemon* as bioindicators for environmental management. Another studies on the phytochemical investigations of members of this genus resulted in the identification of *C*-methyl flavonoids, triterpenoids and phloroglucinol derivatives [6]. The present study was carried out to investigate the chemical composition and fumigant toxicity of essential oil from *C.viminalis* against the adults of against *D. melanogaster*.

MATERIAL AND METHODS

Plant collection

Callistemon viminalis leaves were collected from Al-Jadiriya Districts/ Baghdad /Iraq in the month of October-November 2015. The taxonomic identification of the plant materials was confirmed by the botanist in plant laboratory/ Biology department/ college of Science/ Baghdad University. The leaves were washed with, air dried in the shade for a week with continuous stirring to prevent it from rotting. The dried leaves were pulverized to a powder using electrical grinder. The powders were preserved in a glass jar and stored at 4°C until used.

Extraction and chemical analysis of the essential oil

Essential oil was extracted from the plant sample using a modified Clevenger-type apparatus. 300g of leaves powder mixed with 500 ml distilled water poured into the Clevenger, evaporate at 100°C, hydrodistilled for 3 to 4 hours. Anhydrous sodium sulfate was used to eliminate water after extraction [9, 10]. The leaves of *C.viminalis*, on hydrodistillation, gave 0.41 % of oil on a fresh weight basis. The extracted oil was placed in a sealed glass tubes and stored at 4°C for chemical analysis and until used for insect bioassays.

Gas chromatographic (GC) analysis was done in Abn-sinaa at college of science for women/ Baghdad University, using a thermoquest-finnigan on a DB-1 fused-silica capillary column (60m × 0.25mm id., 0.25 µm film thickness). The temperature was programmed to rise to a 50°C and then to 240°C at 5°C/ min. Injector and detector temperatures was 200 and 240°C,

respectively. The detector was a flame ionisation detector and hydrogen was used as a carrier gas at a flow rate of 1.1 ml/ min. Diluted oil (0.5 µL) was injected into the GC [9]. The chemical components of the oils were identified base on the comparison of their retention indices and mass spectra with standards.

Insect rearing

Drosophila melanogaster were acquired from a breeding colony from a laboratory to be cultured. The flies were placed in bottles (80 mL). For the preparation of standard fruit culture media, 100 g of banana fruits were blended and mixed well with 100 mL of liquefied agar. Standard yeast was sprinkled evenly on the top of the food in a single layer to attract the flies (or Just before usage, the food is seeded with yeast, by adding a drop of thick suspension of fresh made yeast solution). Adult flies were transferred into identical, clean bottles approximately every 7 days. For optimal results of culturing these flies, cultures are usually incubated at a constant temperature between 23±2 ºC and 60% R.H. [11]. All experiments were carried out under the same environmental conditions.

Fumigant toxicity bioassays

In order to test the fumigant toxicity of *C. viminalis* essential oil, concentrations 15, 25, 40 and 60 µl/L air of the oil were dissolved in 100 µl acetone, dried in air for 2 min and applied on a filter paper (Whatman No.1) strip measuring 4 × 5 cm that was attached to the lower side of the jars lid. Twenty adults (1-3 days old of undefined sex) of insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical center of 1 L glass jars, which were then sealed with air tight lids. Thus, there was no direct contact between the oil and the insects. In the control jars, only acetone applied on the filter papers [12]. Mortality was determined after 5, 10 and 24 h from commencement of the exposure. Each experiment was replicated four times for each concentration in addition to control. If no leg or antennal movements were observed, insect was considered dead.

Statistical analysis

Probit analysis for determination of LD_{50} was conducted on the bioassay data (total mortality after initial exposure) after correction for control mortality using Abbott's formula [13] followed by log transformation using the log- probit analysis software [14]:

$$Ma\,(\%) = [(Mt - Mc)/(100 - Mc)] \times 100$$

Where Ma is corrected mortality (%), Mt is mortality in the treatment (%), and Mc is mortality in the control (%). Mortality and repellency data were subjected to a one-way ANOVA and followed by the Tukey and Fisher tests respectively.

RESULTS

Fumigant toxicity

The essential oil vapor showed a potent insecticidal activity

Table 1: Fumigant of *C. viminalis* against *D. melanogaster*

Concentration (µl/L)	Insect mortality (%) after		
	5h	10h	24h
15	10.27 (6.0-16.44)	21.02 (15.11-26.72)	33.25 (24.0-35.22)
	6.0	15.11	24.0
	10.22	26.72	35.22
	16.44	19.45	29.64
25	24.73 (17.66-45.06)	37.34 (25.45-50.77)	58.50 (50.71-80.21)
	17.66	25.45	50.71
	45.06	50.77	50
	25	50	80.21
40	35.5 (22.98-51.02)	41.75 (25.0-56.54)	77.25 (47.50-89.32)
	22.98	50	89.32
	25 51.02	25 56.54	75 47.50
60	51.5 (39.24-60.51)	73.25 (47.61-84.33)	97.37 (92.0-100)
	60.51	84.33	92.0
	39.24 50	75 47.61	100 100

Table 2: Fumigant toxicity of *C. viminalis* against *D. melanogaster*

Time	LC_{50} (%)	Slope (± SE)	Intercept	R^2
After 5 h	56.573	2.111 ± (0.387)	1.299	0.993
After 10 h	37.223	2.127 ± (0.223)	1.659	0.951
After 24 h	20.579	3.5993± (0.542)	0.273	0.979

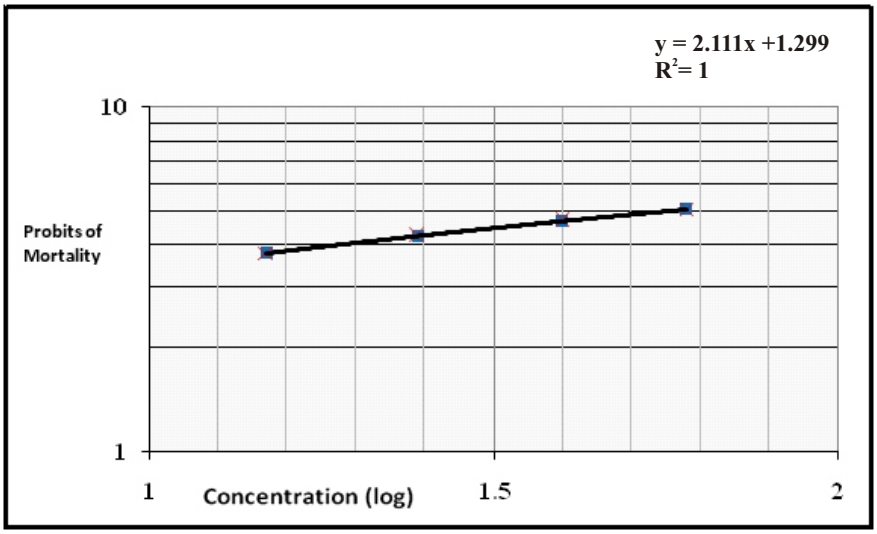

Figure 1: Mortality rate for adults of *D.melanogaster* treated with extracts of *C. viminalis* after 5 hours

against the adults of *D. melanogaster*. The maximum mortality of 51.5, 73.25 and 97.37 % was achieved at the concentrations of 60 µl/L after 5, 10 and 24 h., respectively (Table.1). Also, the least concentration of 15 µl/L showed the moderate mortality of 21.02 and 33.25 % after 10 and 24 h from exposure.

The probit statistics, estimate of LC_{50} for 5, 10 and 24 hours after treatment are presented in Table 2. A difference in adult mortality according to time was observed at all concentrations of essential oil treatments. On the bases of LC_{50} values, laboratory data have indicated a positive correlation between these values and exposure time. Results in table 2 and figure (1,2,3) showed that the LC_{50} values after 5h was 56.573%, while it was 37.223% after 10h. and reached to 20.579 % after 24 h.

Chemical constituents of *C. Viminalis*

The results of gas chromatography (GC) analysis indicated that the essential oil extracted from the leaves of *C. viminalis* contain 1,8-cineole (57.35%), *α*-pinene (10.20%) , aspartic (12.30%) and palmitic acid (15.65%).

DISCUSSION

Results revealed that the tested concentrations were toxic against *D. melanogaster*, and mortality percentages were directly proportional to the essential oil concentrations and time after treatment.

Many essential oils extracted from different plant spices have already been screened for toxicity as potential insecticide against different insect. *Eucalyptus camaldulensis* and *C. viminalis* essential oils were tested for fumigant toxicity against adults of *T.confusum* either with or devoid of wheat grains [15]. The essential oil of *Satureja hortensis* L. showed fumigant toxicity against larvae of *E. kuehniella* [16]. Essential oil derived from *Pistacia lentiscus* L. is known to have toxic effects on *E. kuehniella* [17]. The essential oil from oregano, *Origanum onites* L., and savory, *Satureja thymbra* L. has also been reported to have fumigant toxicity toward adults of *E. kuehniella* [18].

Previous studies on the essential oils of *C. viminalis* from Australia, Egypt, India, Pakistan and Reunion Island have been reported that 1,8-Cineole (47.9-82.0%) was the predominant constituent of the oils. Other significant components included α-pinene, β-pinene, myrcene, limonene, linalool and menthyl acetate [19- 22]. Also, these studies from different region have been showed that there are differences in the constituents and yield of the oils, which might be attributed to difference in geographical and environmental conditions.

Results on our sample species from Baghdad showed quantitative differences. In the essential oil of *C. viminalis* from Egypt, 1,8-cineole represented 47.9%% of the total oil and in the South African species, it was 83.2%, while in Baghdad sample, it was 57.35%. GC and GCMS analysis of the oil resulted in the identification of 42 constituents, representing 99.5% of the oil. 1,8-Cineole (61.7%), *α*-pinene (24.2%) and menthyl acetate (5.3%) were the major components [20].

CONCLUSION

Our research concluded that the essential oil extracted from *C. viminalis* is a promising option for managing *D. melanogaster* being natural insecticide to reduce environmental and public health hazards resulting the use of chemicals. At the same time, these natural products are considered fully biodegradable, do not

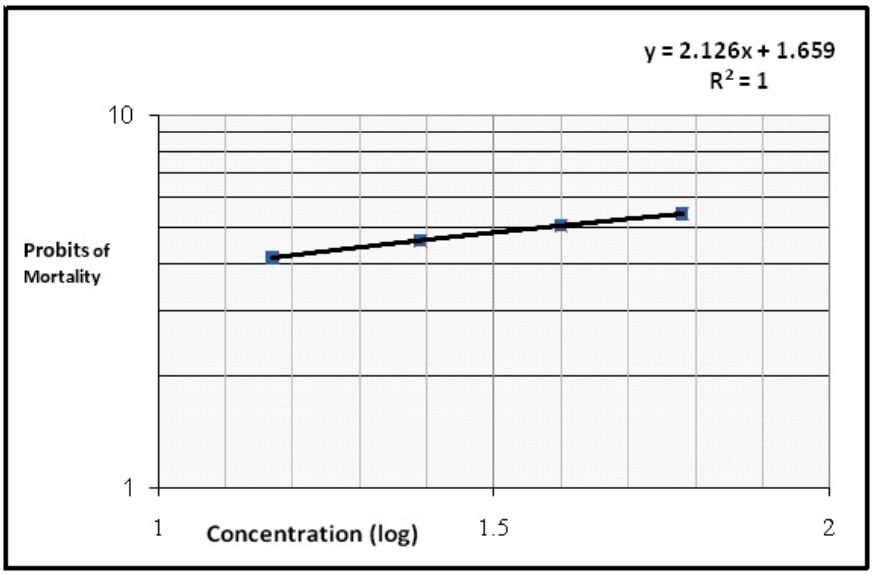

Figure 2: Mortality rate for adults of *D.melanogaster* treated with extract of *C. viminalis* after 10 hours

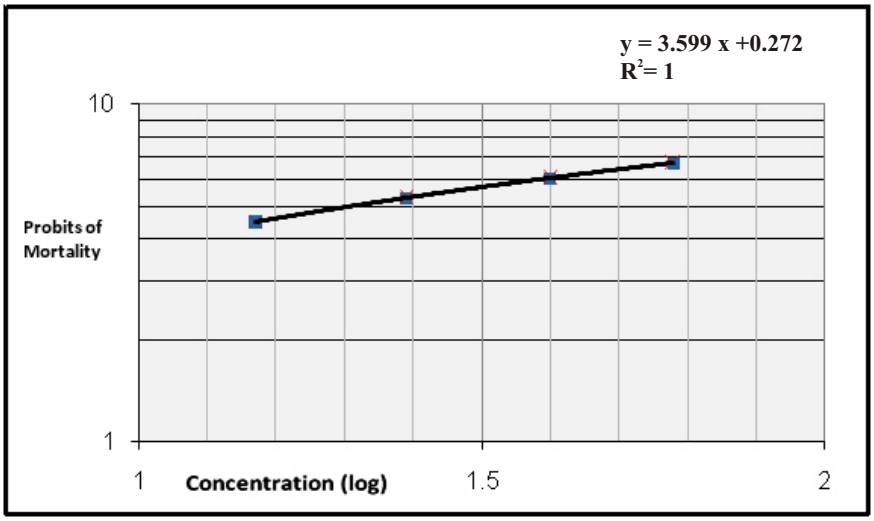

Figure 3: Mortality rate for adults of *D.melanogaster* treated with extracts of *C. viminalis* after 24 hours

leave toxic residues and can pose lesser risks to human health and the environment. Thus, it is candidate to further investigate to improve their efficacy.

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